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Thermal Stress as a Critical Factor in the Viability and Duration of Spittlebug Eggs

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Abstract: The spittlebug *Mahanarva spectabilis* (Distant, 1909) (Hemiptera: Cercopidae) is an important pest that causes significant losses in the production of forage crops for cattle feed. Information on the thermal requirements of this insect during the egg stage is crucial in assessing the interaction between insects and forage. The aim of this research was to evaluate the effects of constant and oscillating (diurnal/nocturnal) temperatures on the viability of *M. spectabilis* eggs and the duration of the egg stage. Temperatures of 20 °C to 30 °C were ideal for the development of this insect pest, resulting in greater viability and faster development of the embryos. In addition, it should be noted that a variation of up to 8 days is feasible for synchronizing the phenological stages of the forage plants and the eggs to be laid on these plants when subjected to 30 °C (16.6 days) or 20 °C (25.7 days) without significantly altering the viability of the eggs. Notably, a temperature oscillation of 25 °C during the day and 15 °C at night increased the viability of the eggs after exiting diapause. These results are essential for the rearing of *M. spectabilis* in the laboratory, allowing for the supply of eggs for experiments and contributing to advances in studies aimed at developing effective integrated management strategies for this pest.

Keywords: insect; diapause; *Mahanarva spectabilis*; biology; pastures

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1. Introduction

Spittlebugs are insect pests that limit the yields of the main forage crops used as food for cattle [1–3]. The global losses caused by the herbivory of spittlebugs are estimated to amount to USD 2.1 billion per year [4], and they are caused mainly by insects in the adult stage [5]; however, the survival strategies of these insects are best observed in the egg stage, for which diapause begins during the dry season [6].

After reaching adulthood, *Mahanarva spectabilis* (Distant, 1909) (Hemiptera: Cercopidae) feeds by sucking the sap of forage plants, and, in general, each female lays an average of nearly 100 eggs [7]. There are four stages of embryogenic development in the eggs. During the rainy season, the development of an *M. spectabilis* egg takes about 20 days [6,8]; and in the dry season, the survival of *M. spectabilis* eggs requires a mechanism known as diapause [6], which is a process where development is delayed, cell division is slowed or stopped, and stress tolerance is enhanced [9]. As a consequence, the organism enters in a physiological state that allows its survival under unfavorable conditions.

Temperature is undoubtedly the main abiotic determinant of insect development and survival [10]. Insect eggs in the field or in the laboratory need to be protected from external risks that could prevent the corresponding insects from hatching. A variety of hexapod eggs appear to be tolerant to such risks [11]. Under optimal conditions, in Brazil, the development of *M. spectabilis* eggs takes approximately 20 days [6,8]. However, environmental conditions are not always optimal for egg development, and survival requires a mechanism

known as diapause. Knowing the main biological aspects of spittlebug eggs is important not only for establishing the critical factors for survival at this stage and, consequently, their population fluctuations but also for maintaining these cercopids in the laboratory, allowing for the provision of eggs for experiments and contributing to advances in studies aimed at developing effective integrated management strategies for this pest.

Although large-scale rearing of *Mahanarva fimbriolata* (Stål, 1854) has been successful, the existence of eggs in diapause has been an obstacle to the constant maintenance of this pest throughout the year [12]. The last generation occurs in April/May, coinciding with the start of the dry season and mild temperatures, and the insects remain in diapause until the next rainy season [13,14]. Using thermal shocks, varying the photoperiod, and maintaining high humidity to break diapause have already been studied [8,14,15]. The interruption of development offers significant advantages to insects, allowing them to use only small portions of the year for growth and reproduction. By entering diapause, insects can avoid adverse periods, and by detecting suitable environmental signals, they can resume active development when favorable conditions return. The ability to manipulate diapause represents a significant challenge with respect to the mass rearing and storage of insects [16].

The greatest obstacle to progress in studies on spittlebugs is the difficulty of rearing large numbers of insects throughout the year to infest the plants under study, a problem that can be prevented by diapause. In addition, the storage temperature of the eggs should be determined to synchronize eggs at the final embryonic stage (required for insertion into plants in studies of insect-versus-forage interactions), and the plants should be at the appropriate stage for infestation. Hence, the aims of this research were as follows: (i) Determine the period(s) in which the eggs need temperature oscillation (diurnal/nocturnal) to promote the breaking of diapause to make the insect available all year round for research purposes, and (ii) evaluate the effects of five constant temperatures on the embryonic development and viability of *M. spectabilis* eggs to ensure synchronism between the phenological stage of the corresponding plant and the embryo and meet the methodological criteria for studying insect–forage interactions.

2. Results

2.1. Temperature as a Critical Factor

At the five temperatures studied, it was found that the shortest embryonic stage and total egg stage of *M. spectabilis* were found at temperatures of 20, 25, and 30 °C. At the highest temperatures, 25 and 30 °C, the insect's embryonic development was significantly different, regardless of the egg stage analyzed, with an average of approximately 5 days for the initial stage (S1 and S2), 3 days for the two subsequent stages (S3 and S4), and 17 days for the total egg stage (Table 1). The average development time of the different embryonic stages and the total egg stage decreased as the thermal conditions significantly increased, following a second-degree equation (Figure 1A–E). The viability of *M. spectabilis* eggs at the different stages was greatest when the eggs were kept at 20, 25, and 30 °C and did not differ from each other (Table 2). At the lowest temperatures, 10 and 15 °C, the viability was less than 15% and 55%, respectively, which means that few were able to reach stage S4 and subsequently hatch (Table 2). The viability of the embryonic stages stabilized significantly at 20 °C and above, following a second-degree equation. This same equation was adjusted for the total egg stage (Figure 2A–E).

Using these equations, it was possible to determine that 24 ± 1 °C and 26 ± 1 °C were the optimal temperatures, for they provided higher viability and the shortest duration of the embryonic stage and total egg phase, respectively.

Table 1. Duration (days) (±EP) of the embryonic stage (S1 to S4) and total egg stage of *M. spectabilis* specimens subjected to constant temperatures (10, 15, 20, 25, and 30 ± 1 °C), 70% ± 10% RH, and 12 h photophase.

	Temperatures						
Stadium	**10 °C**	**15 °C**	**20 °C**	**25 °C**	**30 °C**	***F***	***p***
S1	35.18 ± 1.72 d	29.71 ± 0.88 c	10.08 ± 0.21 b	5.61 ± 0.10 a	5.22 ± 0.13 a	268.41	<0.001
S2	8.60 ± 1.96 b	21.93 ± 0.71 c	8.60 ± 0.21 b	5.31 ± 0.15 a	5.00 ± 0.20 a	53.58	<0.001
S3	5.40 ± 0.69 b	10.13 ± 1.03 c	4.01 ± 0.16 ab	3.20 ± 0.13 ab	2.83 ± 0.21 a	27.40	<0.001
S4	4.93 ± 1.02 b	10.50 ± 0.69 c	3.01 ± 0.11 a	3.85 ± 0.10 a	3.59 ± 0.15 a	31.08	<0.001
Total egg stage	54.86 ± 1.30 c	71.45 ± 2.37 d	25.70 ± 0.43 b	17.91 ± 0.22 a	16.58 ± 0.25 a	369.49	<0.001

Averages followed by the same letter in the same line did not differ according to Tukey's test ($p < 0.01$).

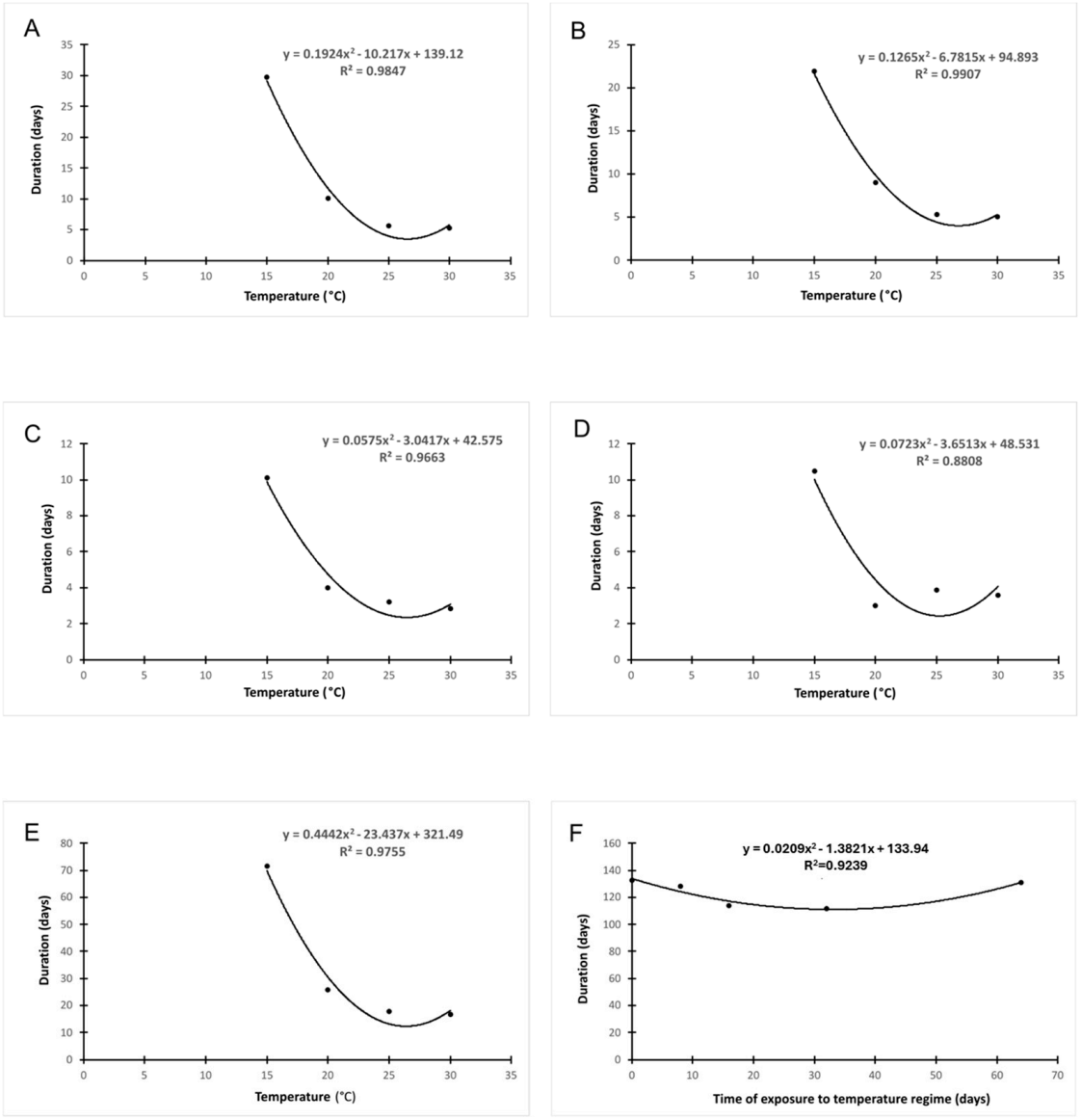

Figure 1. Duration (in days) of embryonic stages S1 (**A**), S2 (**B**), S3 (**C**), and S4 (**D**) and total egg stage (**E**) of *M. spectabilis* specimens subjected to constant temperatures (10, 15, 20, 25, and 30 ± 1 °C), an RH of 70% ± 10%, and a 12 h photophase, and durations (in days) of total egg stage of insects after exposure to different periods of temperature oscillation (diurnal/nocturnal) (**F**).

Table 2. Viability (%) (±EP) of *M. spectabilis* in the embryonic stage (S1 to S4) and total egg stage after being subjected to constant temperatures (10, 15, 20, 25, and 30 ± 1 °C), 70% ± 10% RH, and 12 h photophase.

	Temperatures						
Stadium	**10 °C**	**15 °C**	**20 °C**	**25 °C**	**30 °C**	***F***	***p***
S1	28.12 ± 4.39 a	83.33 ± 4.95 b	81.25 ± 3.92 b	88.54 ± 2.41 b	84.37 ± 3.49 b	41.53	<0.001
S2	20.83 ± 3.87 a	63.54 ± 5.85 b	72.91 ± 3.01 b	77.08 ± 4.31 b	72.91 ± 3.38 b	30.60	<0.001
S3	15.62 ± 2.72 a	60.41 ± 5.72 b	72.91 ± 3.01 b	75.91 ± 4.35 b	70.83 ± 3.55 b	38.26	<0.001
S4	15.62 ± 2.72 a	55.20 ± 6.05 b	71.87 ± 3.13 bc	73.95 ± 4.98 c	68.75 ± 3.92 bc	31.44	<0.001
Total egg stage	15.62 ± 2.71 a	57.29 ± 5.64 b	71.87 ± 3.13 bc	76.04 ± 5.43 c	68.75 ± 3.92 bc	32.34	<0.001

Averages followed by the same letter in the same line did not differ according to Tukey's test ($p < 0.01$).

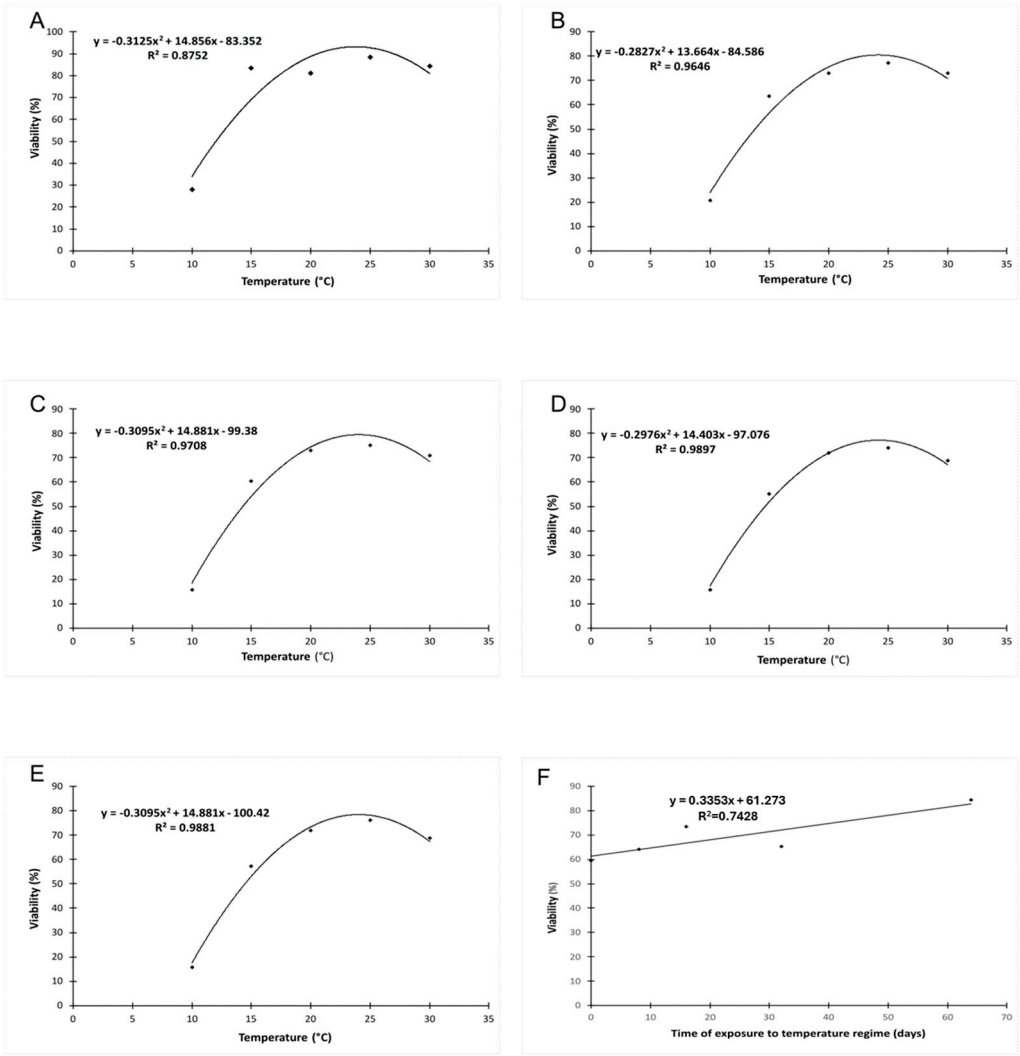

Figure 2. Viability (%) of embryonic stages S1 (**A**), S2 (**B**), S3 (**C**), and S4 (**D**) and total egg stage (**E**) of *M. spectabilis* specimens subjected to constant temperatures (10, 15, 20, 25, and 30 ± 1 °C), 70% ± 10% RH, and 12 h photophase, and viability (%) of total-egg-stage insects after exposure to different periods of temperature oscillation (diurnal/nocturnal) (**F**).

2.2. Duration of Exposure to Temperature Oscillation (Diurnal/Nocturnal) as a Critical Factor

The total egg stage of *M. spectabilis* became significantly shorter as the temperature oscillation period increased to 32 days and remained the same from then on, following a second-degree equation, with a variation in duration from 112 to 133 days (Figure 1F).

Egg viability was significantly greater when the eggs were kept for 64 days under day/night temperature oscillation conditions, with 84.4% of the eggs hatching. The lowest significant viability was found for the eggs kept at a constant temperature (59.4%). Increasing the temperature oscillation period led to a significant increase in egg viability, following a first-degree equation (Figure 2F).

3. Discussion

Identifying the factors that determine the viability and duration of the embryonic period of the spittlebug *M. spectabilis* is important for rearing this insect in the laboratory and obtaining eggs under the same embryonic conditions; infesting forage plants to evaluate insect–plant interactions in the laboratory and greenhouse; and predicting population fluctuations of this cercopid throughout the year in the field.

Temperature is often the primary abiotic determinant of insect development and survival [10]. Although insects can survive and reproduce within a range of temperatures, their performance is often worse near their minimum and maximum temperature limits [17]. Therefore, determining the ideal temperature for maintaining these eggs is necessary. In addition, studies on insect and plant interactions require many eggs at the same embryonic stage (S4) because, according to Auad and Carvalho [8], this is the right time to place the eggs on the plants; at this stage, the eggs are close to 100% viable and have a shorter period of exposure to biotic and abiotic factors, thus guaranteeing the hatching of the nymphs and promoting greater reliability in assessing the survival of subsequent stages.

In the present study, the developmental rate of the eggs of specimens of the spittlebug *M. spectabilis* subjected to low temperatures was affected, thus prolonging the embryonic stage and the total egg stage, which leads to worse conditions for the insect's embryonic development. Auad et al. [18] reported that eggs of this species subjected to 28, 24, and 20 °C had embryonic periods of 17.4, 22.3, and 28 days, respectively. Garcia et al. [13] reported the same pattern of embryonic development for *M. fimbriolata* eggs, which were subjected to temperatures of 18, 20, 22, 25, 28, and 30 °C, wherein an increase in the embryonic period was recorded from 18.2 days (30 °C) to 62.3 days (18 °C). Although the impact of temperature on development can vary among species, development time is generally longer at lower temperatures [7] and has similar effects on all insect-life stages in terms of accelerating development [19].

Eggs kept at 20, 25, and 30 °C had ideal viabilities with respect to being kept in a laboratory, with more eggs reaching the hatching stage. In the current study, a value of 24 ± 1 °C was determined to be the optimal temperature because it provided the highest viability. At the lowest temperatures, 10 and 15 °C, the viability was less than 15% and 55%, respectively, which means that few insects managed to reach the S4 stage and subsequently hatch. Garcia et al. [13] reported survival rates of over 68.9% in the range of 18 °C to 30 °C and 0% at 32 °C. For the same species, Garcia et al. [20] reported an egg viability of 81.0% when the eggs were kept at 25 °C.

Taking into account the two parameters, duration and viability, at temperatures of 25 and 30 °C, the total egg stages of *M. spectabilis* lasted an average of 17.9 and 16.6 days, respectively, with average egg viabilities of 76% and 69% and the corresponding insects reaching the hatching stage, respectively. These conditions were the most viable for keeping the eggs in the laboratory because, in just a few days, it is possible to obtain a greater quantity of eggs, which will reach the stage required to meet the methodological standard for studies of insect–plant interactions. Notably, at a temperature of 20 °C, the average duration of the total egg stage was 25.7 days, with an average viability (S1 egg to nymph hatching) of 72%. This means that *M. spectabilis* eggs kept at this temperature can be maintained for almost a month without compromising their viability, unlike those kept at 25 and 30 °C, allowing for flexibility if more time is needed to synchronize the phenological stages of plants and insects for infestation experiments with the insect pest *M. spectabilis*. Notably, at the two lowest temperatures (10 and 15 °C), although it is possible to extend the embryonic period, as the insect takes an average of 54 and 71 days, respectively, to

hatch, viability was below 15% (10 °C) and 55% (15 °C), reinforcing the fact that those kept at 10 °C did not develop within a month, and this temperature was changed to 25 °C in an attempt to extend the cycle without compromising viability.

According to Denlinger [21] and Koštál [22], some animals enter a state of dormancy, in which development slows, metabolic activity reduces, nutrient reserves increase, and the ability to withstand desiccation, hunger, and cold improves. These results corroborate our findings, demonstrating that when insects are subjected to unfavorable conditions, they tend to enter a state of diapause, preserving their eggs for the hatching of nymphs in favorable environmental conditions. An understanding of the mechanisms that regulate diapause and determine the pattern of its occurrence in insect pest populations is an important tool in their management [23]. Moreover, knowledge of the events that can cause changes in the metabolism and thus lifecycle of spittlebugs is essential for understanding the seasonal fluctuations in their populations [14,24]. Nearly all eggs laid by *M. spectabilis* during the wet season are not in diapause, but eggs in diapause become predominant when the wet season ends [6]. Heat shock can directly influence the time spittlebug eggs spend in diapause [14]. The cited authors reported that the greater the exposure to heat shock, the greater the synchronization of egg hatching. However, considering that the difference between the control treatment and the treatment in which insects were kept for 64 days was 21 days until the nymphs hatched in the present research, it can be inferred that even with a longer duration of exposure to temperature oscillation, the insects remained in the egg stage for 112 days (3.7 months), corresponding to a duration seven times longer when considering the embryonic period of eggs not in diapause based on the results obtained by Auad and Carvalho [8] and Auad et al. [6].

The ability to enter diapause enables insects to survive adverse conditions, take advantage of seasonally fluctuating resources, diversify in tropical habitats, and colonize temperate and polar regions. The research community now broadly recognizes diapause as a dynamic process rather than a static state, a perspective supported by studies on insects and other organisms [25–34]. Diapaused eggs remain in the egg phase for an average of 154.5 days for *M. spectabilis* [6], 196.2 days for *Aeonolamia varia* (Fabricius, 1787) [35], 241 days for *Deois schach* (Fabricius, 1787) [36], and 288 days for *Deois flavopicta* (Stål, 1854) [37]. For this reason, despite the increase in viability with the increase in the temperature oscillation period observed in the present study, the period during which the eggs remained in diapause is still long. Similarly, a reduction in the time the eggs spent in diapause was not possible due to the constant humidity under which the eggs were kept [8] and changes in photoperiods [15]. Thus, during the rainy season, harmful population levels are often reached. This will also be an important period for studies with these insect pests in the laboratory and greenhouse. Notably, after the decision to induce egg diapause was made, the abiotic factors mentioned above were not decisive in reducing the period of diapause immediately.

4. Materials and Methods

4.1. Bioassay 1. Constant Temperatures as a Critical Factor in the Viability of Spittlebug Eggs and the Duration of the Egg Stage

Three hundred adult *M. spectabilis* specimens were collected with an entomological net in the experimental field of Embrapa Gado de Leite in Coronel Pacheco, Minas Gerais (MG), Brazil. These were taken to the entomology laboratory at Juiz de Fora, MG, Brazil, and placed in an acrylic cage (30 × 55 × 30 cm), using *Cenchrus purpureus* (Schumach.) Morrone as a substrate for feeding.

Moistened hydrophilic gauze was added to the basal part of the cage and to the base of the plant, which served as a substrate for oviposition. After 4 days, the gauze was subjected to jets of water using a sieve (400 mesh), which retained the eggs.

The eggs, which were in the first embryonic stage (S1), were individually placed in microtiter plates with 96 compartments. Each compartment was lined with filter paper moistened daily with a 1% copper sulfate solution to combat possible fungal and bacterial

contamination and seeded with one *M. spectabilis* egg. The eggs were placed in BOD-type climate chambers at 10, 15, 20, 25, and 30 ± 1 °C, ±70% RH, and a 12 h photophase throughout the period, except for those kept at 10 °C, which, as they did not show embryonic development in the first 30 days, were then kept at 25 °C.

The duration and viability of embryonic development at S1, S2, S3, and S4 and total egg stage were assessed daily, following the criteria established by Peck [38], where each stage (S) has specific attributes, such as the appearance of the hatching line (S1), the black color of the operculum becoming evident (S2), exposure of the black surface of the operculum (S3), and the exhibition of red ocelli and abdominal spots by the embryo (S4).

4.2. Bioassay 2. The Time of Exposure to Temperature Oscillation as a Critical Factor in Reducing the Diapause Period of M. spectabilis Eggs

Two hundred adult *M. spectabilis* specimens were collected with an entomological net in April, which corresponded to the third peak occurrence of this insect pest, in the experimental field of Embrapa Gado de Leite in Coronel Pacheco, MG, Brazil. They were taken to the entomology laboratory at Juiz de Fora, MG, Brazil, and placed in an acrylic cage (30 × 55 × 30 cm), using *C. purpureus* as a substrate for feeding. The eggs were obtained using the methodology of the previous bioassay and placed in BOD-type chambers at 25 ± 1 °C and 70 ± 10% RH with a 12 h photophase.

The discrimination between eggs in diapause and those not in diapause was performed during the embryonic period, during which, according to Castro et al. [39], those that remained in the egg phase for more than 30 days were considered to be in diapause, and those that did not were not considered to be in diapause.

The eggs in diapause were individually placed in microtiter plates with 192 compartments (each microtiter plate had 96 compartments). Each compartment was lined with filter paper moistened daily with a 1% copper sulfate solution to combat possible fungal and bacterial contamination and seeded with one *M. spectabilis* egg. The eggs were kept in BOD-type chambers, with a humidity of 70 ± 10% and a photophase of 12 h, for periods of 8, 16, 32, and 64 days with temperature oscillation during the day (25 °C) and night (15 °C), following the breakage criterion proposed by Sujii et al. [14] for *D. flavopicta*. The temperature of 15 °C was selected since it was the lowest during the dry season in the region where the eggs were collected. The oscillation from 25 °C to 15 °C was equivalent to 96, 192, 38,4 and 768 h at 15 °C. After each of the above periods, the eggs were stored at 28 °C. In the control treatment, the eggs were always kept at 28 °C, which is a favorable temperature for the development of *M. spectabilis*.

The viability and duration of *M. spectabilis* eggs in diapause subjected to different temperature oscillation periods were evaluated daily.

4.3. Statistical Analysis

The experiments were conducted using a completely randomized design, and each treatment was repeated 12 times, with 8 eggs per repetition (bioassay 1) or 16 eggs per repetition (bioassay 2). The data obtained did not meet normality assumptions related to the residuals and homogeneity of variances (Shapiro–Wilk test, $p < 0.01$). A generalized linear model (GLM), followed by analysis of variance (ANOVA), was used to verify whether there were significant differences in the duration and viability of each embryonic stage (S1 to S4) and the total egg stage at constant temperatures (Bioassay 1) and in the temperature oscillation period (day and night) to alter the duration and viability of diapause eggs (Bioassay 2), and the means were compared via Tukey's test ($p < 0.01$) and polynomial regression analysis. The analyses were carried out via SAS v.9.0 software [40].

5. Conclusions

Notably, a variation of up to 8 days is feasible for synchronizing the phenological stages of forage plants and the embryonic stage of *M. spectabilis* eggs required for studies on the interaction between insects and plants, and varying the temperature from 30 °C

(16.6 days) to 20 °C (25.7 days) does significantly alter the viability of these eggs. Temperature oscillation (day and night) cannot reduce the duration these eggs spend in diapause immediately, although it does increase their viability.

6. Patents

The authors declare that there are no patent registrations associated with this work.

Author Contributions: M.D.: research, writing—review and editing; L.A.C.: research, statistical analysis, and writing—review and editing; A.M.A.: conceptualization, data curation, funding acquisition, research, methodology, project management, statistical analysis, and writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The datasets generated or evaluated during this study are available from the first author upon reasonable request.

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Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Alvarenga, R.; Auad, A.M.; Moraes, J.C.; da Silva, S.E.B.; Rodrigues, B.S. Tolerance to Nymphs and Adults of *Mahanarva spectabilis* (Hemiptera: Cercopidae) by Forage Plants in Fertilized Soils. *Pest Manag. Sci.* **2019**, *75*, 2242–2250. [CrossRef] [PubMed]
2. Alvarenga, R.; Auad, A.M.; Moraes, J.C.; Silva, S.E.B.; Rodrigues, B.S.; Silva, G.B. Spittlebugs (Hemiptera: Cercopidae) and their Host Plants: A Strategy for Pasture Diversification. *Appl. Èntomol. Zool.* **2017**, *52*, 653–660. [CrossRef]
3. Auad, A.M.; Simões, A.D.; Pereira, A.V.; Braga, A.L.F.; Sobrinho, F.S.; Lédo, F.J.d.S.; Paula-Moraes, S.V.; Oliveira, S.A.; Ferreira, R.B. Seleção de Genótipos de Capim-Elefante Quanto a Resistência ã Cigarrinha-Das-Pastagens. *Pesq. Agropec. Bras.* **2007**, *42*, 1077–1081. [CrossRef]
4. Thompson, V. Associative Nitrogen Fixation, C_4 photosynthesis, and the Evolution of Spittlebugs (Hemiptera: Cercopidae) as Major Pests of Neotropical Sugarcane And Forage Grasses. *Bull. Èntomol. Res.* **2004**, *94*, 189–200. [CrossRef]
5. Resende, T.T.; Auad, A.M.; Fonseca, M.G. How Many Adults of *Mahanarva spectabilis* (Hemiptera: Cercopidae) Should Be Used for Screening *Brachiaria ruziziensis* (Poales: Poaceae) Resistance? *J. Econ. Èntomol.* **2014**, *107*, 396–402. [CrossRef]
6. Auad, A.; Simões, A.; Leite, M.; da Silva, S.; dos Santos, D.; Monteiro, P. Seasonal Dynamics Of Egg Diapause In *Mahanarva spectabilis* (Distant, 1909) (Hemiptera: Cercopidae) On Elephant Grass. *Arq. Inst. Biol.* **2011**, *78*, 325–330. [CrossRef]
7. Fonseca, M.G.; Auad, A.M.; Resende, T.T.; Hott, M.C.; Borges, C. How will *Mahanarva spectabilis* (Hemiptera: Cercopidae) Respond to Global Warming? *J. Insect Sci.* **2016**, *16*, 32. [CrossRef] [PubMed]
8. Auad, A.; de Carvalho, C. Desenvolvimento e viabilidade de ovos de *Mahanarva spectabilis* (Distant, 1909) (Hemiptera: Cercopidae) submetidos a diferentes regimes de hidratação. *Arq. Inst. Biol.* **2009**, *76*, 711–714. [CrossRef]
9. King, A.M.; MacRae, T.H. Insect Heat Shock Proteins During Stress and Diapause. *Annu. Rev. Èntomol.* **2015**, *60*, 59–75. [CrossRef]
10. Bale, J.S.; Masters, G.J.; Hodkinson, I.D.; Awmack, C.; Bezemer, T.M.; Brown, V.K.; Butterfield, J.; Buse, A.; Coulson, J.C.; Farrar, J.; et al. Herbivory in Global Climate Change Research: Direct Effects of Rising Temperature on Insect Herbivores. *Glob. Change Biol.* **2002**, *8*, 1–16. [CrossRef]
11. Romani, R.; Isidoro, N.; Bin, F. Antennal Structures Used in Communication by Egg Parasitoids. In *Egg Parasitoids in Agroecosystems with Emphasis on Trichogramma*; Springer: Dordrecht, The Netherlands, 2010; pp. 57–96.
12. De Bortoli Alves, D. *Efeito do Ácido Clorídrico Sobre a Viabilidade de Ovos de Mahanarva fimbriolata*; Repositório UFV: Viçosa, Brazil, 2017; p. 17.
13. Garcia, J.F.; Botelho, P.S.M.; Parra, J.R.P. Biology and Fertility Life Table of *Mahanarva fimbriolata* (Stål) (Hemiptera: Cercopidae) in Sugarcane. *Sci. Agric.* **2006**, *63*, 317–320. [CrossRef]
14. Sujii, E.R.; Garcia, M.A.; Fontes, E.M.G.; Carvalho, V. Efeito Da Temperatura e Umidade Sobre o Término Da Diapausa de Ovos e Densidade Populacional Da Cigarrinha-Das-Pastagens, *Deois flavopicta* (Stal) (Homoptera: Cercopidae). *An. Soc. Entomol. Braz.* **1995**, *24*, 465–478. [CrossRef]
15. Simões, A.D.; Lima, E.R.; Auad, A.M.; Resende, T.T.; Leite, M.V. Development of the Spittlebug *Mahanarva fimbriolata* under Varying Photophase Conditions. *J. Insect Sci.* **2013**, *13*, 105. [CrossRef]
16. Colinet, H.; Boivin, G. Insect Parasitoids Cold Storage: A Comprehensive Review of Factors of Variability and Consequences. *Biol. Control.* **2011**, *58*, 83–95. [CrossRef]

17. Haddad, M.L.; Parra, J.R.P.; Moraes, R.C.B. *Métodos Para Estimar Os Limites Térmicos Inferior e Superior de Desenvolvimento de Insetos*; FEALQ; Repositório USP: Piracicaba, Brazil, 1999; p. 29.
18. Auad, A.M.; Carvalho, C.A.; Resende, T.T. Impacto Da Temperatura No Período Embrionário de *Mahanarva spectabilis* (Distant, 1909) e *Deois schach* (Fabricius, 1787) (Hemiptera: Cercopidae). In Proceedings of the Congresso Latino Americano De Ecologia, São Lourenço, Brazil, March 2009.
19. Amarasekare, P.; Sifuentes, R. Elucidating the Temperature Response of Survivorship in Insects. *Funct. Ecol.* **2012**, *26*, 959–968. [CrossRef]
20. Garcia, J.F. Técnica de Criação e Tabela de Vida de *Mahanarva fimbriolata* (Stål, 1854) (Hemiptera: Cercopidae). Master's Thesis, Escola Superior de Agricultura "Luis de Queiroz", Universidade de São Paulo, Piracicaba, Brazil, 2002. [CrossRef]
21. Denlinger, D.L. Dormancy in Tropical Insects. *Annu. Rev. Èntomol.* **1986**, *31*, 239–264. [CrossRef]
22. Koštál, V. Eco-physiological phases of insect diapause. *J. Insect Physiol.* **2006**, *52*, 113–127. [CrossRef]
23. Tauber, M.J.; Tauber, C.A.; Nyrop, J.P. Soil Moisture and Postdormancy Emergence of Colorado Potato Beetles (Coleoptera: Chrysomelidae): Descriptive Model and Field Emergence Patterns. *Environ. Èntomol.* **1994**, *23*, 1485–1496. [CrossRef]
24. Sujii, E.R.; Garcia, M.A.; Fontes, E.M.G.; Pires, C.S.S.; O'neil, R.J. Effects of Meteorological Variation on Mortality in Populations of the Spittlebug *Deois flavopicta* (Homoptera: Cercopidae). *Environ. Èntomol.* **2002**, *31*, 299–305. [CrossRef]
25. Danilevskii, A.S. *Photoperiodism And Seasonal Development of Insects*; Oliver & Boyd Ltd.: Edinburgh, UK, 1965; p. 283.
26. Danilevsky, A.S.; Goryshin, N.I.; Tyshchenko, V.P. Biological Rhythms in Terrestrial Arthropods. *Annu. Rev. Èntomol.* **1970**, *15*, 201–244. [CrossRef]
27. Taylor, L.R.; Tauber, M.J.; Tauber, C.A.; Masaki, S. Seasonal Adaptations of Insects. *J. Anim. Ecol.* **1987**, *56*, 374. [CrossRef]
28. Denlinger, D.L. Regulation of Diapause. *Annu. Rev. Èntomol.* **2002**, *47*, 93–122. [CrossRef] [PubMed]
29. Denlinger, D.L. Molecular regulation of insect diapause. In *Cell and Molecular Response to Stress*; Elsevier: Amsterdam, The Netherlands, 2000; pp. 259–275.
30. Hodek, I. Controversial aspects of diapause development. *Eur. J. Èntomol.* **2002**, *99*, 163–173. [CrossRef]
31. Hodek, I. Dormancy. In *Ecology of Coccinellidae*; Springer: Dordrecht, The Netherlands, 1996; pp. 239–318.
32. Hodek, I.; Hodková, M. Multiple Role of Temperature During Insect Diapause: A Review. *Èntomol. Exp. Appl.* **1988**, *49*, 153–165. [CrossRef]
33. Danks, H.V. Insect Life-Cycle Polymorphism: Current Ideas and Future Prospects. In *Insect Life-Cycle Polymorphism*; Springer: Dordrecht, The Netherlands, 1994; pp. 349–365.
34. Danks, J.H.; End, L.J. Processing Strategies for Reading and Listening. In *Comprehending Oral and Written Language*; Brill: Dordrecht, The Netherlands, 1987; pp. 271–294.
35. Morales, J. Egg Diapause and Pest Management of *Aeneolamia varia* (Homoptera: Cercopidae) in Venezuela. *Environ. Èntomol.* **1993**, *22*, 1092–1095. [CrossRef]
36. Kalvelage, H.; Buzzi, Z.J. Ciclo de Vida da Cigarrinha-Das-Pastagens *Deois* (*Pandysia*) *schach* (Fabricius, 1787) (Homoptera, Cercopidae), a Duas Temperaturas. *An. Soc. Entomol. Bras.* **1986**, *15*, 113–136. [CrossRef]
37. Pacheco, J.M. *Aspectos da Biologia d Ecologia de Deois (Achantodeois) flavopicta (Stal, 1854) na Região de São Carlos*; Repositório UFSCAR: São Carlos, Brazil, 1981; p. 111.
38. Peck, D.C. Distribución Y Reconocimiento Del Salivazo De Los Pastos (Homoptera: Cercopidae) En La Costa Caribe De Colombia. *Pasturas Trop.* **2002**, *24*, 4–15.
39. Castro, U.; Morales, A.; Peck, D.C. Dinámica Poblacional Y Fenología Del Salivazo De Los Pastos *Zulia carbonaria* (Lallemand) (Homoptera: Cercopidae) En El Valle Geográfico Del Río Cauca, Colombia. *Neotrop. Èntomol.* **2005**, *34*, 459–470. [CrossRef]
40. SAS Institute Inc. *SAS 9.0 [Software]*; SAS Institute Inc.: Cary, NC, USA, 2004.